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New cyclodextrin dimers and trimers capable of forming supramolecular adducts with shape-specific ligands

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Abstract

Bridged cyclodextrin dimers and trimers, in which respectively two and three hydrophobic cavities lie in close proximity, display much higher binding affinities and molecular selectivities than do parent cyclodextrins (CDs). By joining β CD units with links inserted at different positions (2-2', 3-2', 6-2' or 6-2'-6'') and interposing spacers of different lengths and shapes, multicavity structures can be synthesized that are precisely tailored to fit specific guest molecules. This enzyme-mimicking strategy can also be used to generate stable supramolecular adducts. A series of CD dimers and trimers was prepared in good yields by carrying out the critical synthetic steps under power ultrasound (US) or microwave (MW) irradiation. Starting from CD azide and acetylenic derivatives we exploited an efficient MW-promoted Huisgen 1,3-dipolar cycloaddition in the presence of Cu(I) salts. The resulting bridged CD derivatives gave stable adducts with magnetic-resonance-imaging contrast agents (MRI CAs) containing gadolinium(III) chelates. These inclusion complexes were found to be 2 to 3 orders of magnitude more stable than those formed by β CD and to be endowed with high relaxivity values.

Introduction

Cyclodextrins (CDs) find numerous important applications in chemical, biological and pharmaceutical technologies.¹ Their employs range from analytical separations,² food manufacturing,³ enzyme mimics,⁴ as drug carriers,⁵ to the delivery of medical diagnostic agents.⁶ They are among the most widely used oligosaccharide hosts for drug complexation, because CD-encapsulated drugs usually have a better bioavailability, a longer half-life in the body, unhindered excretion and no extra toxicity.⁷

Since the pioneer work by Tabushi⁸ and Harada⁹ much effort has been devoted to making CD dimers (bis-CDs) with a variety of functional tethers.¹⁰ Although syntheses and properties of homodimers linked through their primary¹¹ or secondary¹² faces are well documented, few heterodimers and trimers (tris-CDs)¹³ have been reported to date and not much is known about their binding properties. We described improved US- and MW-promoted protocols for β

CD homodimerization through 6-6' and 2-2' ureido- and thioureido-bridges¹⁴ or alkenyl bridges built by cross-metathesis reactions in the presence of 2nd-generation Grubbs catalyst.¹⁵ Very recently we introduced a new, efficient synthetic protocol¹⁶ for preparing homo- and heterodimers of α -, β - and γ CD. It was based on a MW-promoted¹⁷ Huisgen 1,3-dipolar cycloaddition¹⁸ of CD monoazides and CD monoacetylenes resulting in the formation of a 1,2,3-triazole bridge.¹⁹

Owing to problems of selectivity, efficiency and purification, monosubstitution and disubstitution of CDs still remain challenging targets. We found that non-conventional techniques (US or MW irradiation) could markedly improve several CD functionalizations, to great advantage in terms of yields and reaction times.²⁰⁻²²

Mono-*O*-propargyl-CDs and monoazido-deoxy-CDs proved to be versatile building blocks for the construction of all types of homo- and heterodimers, whether joined 'head-to-head' or 'head-to-tail'. Selective mono- and disubstitutions to provide azido derivatives could usually be achieved *via* monotosylation²⁰ as described in our improved protocols that afforded the 3- and 6-monoazido as well as the 6,6'-diazido derivative.²² The latter is a suitable building block for preparing CD trimers.

Mono-*O*-propargylation on the secondary face was not a straightforward procedure; protective groups had to be inserted at C-6 and strict operative conditions adhered to in order to minimize time-consuming chromatographic purifications (Scheme 1). An alternative strategy for the synthesis of dimers employed a spacer molecule bearing two azido groups or two acetylenic moieties that reacted with suitably monosubstituted CDs (Scheme 2).

The key step in the present synthetic protocol is the Huisgen 1,3-dipolar cycloaddition of azides and alkynes, that under catalysis with copper(I) salts react regioselectively to give 1,4-disubstituted 1,2,3-triazoles. It is experimentally straightforward and widely applicable because it shows a high tolerance toward many functional groups and proceeds to completion in a large variety of solvents including aqueous *t*-butanol and DMF. The catalyst is usually prepared *in situ* by reduction of Cu(II) salts (that are less expensive and often purer than Cu(I) salts), usually with L-ascorbic acid or sodium L-ascorbate. When this cycloaddition was carried out under MW irradiation, the reaction time was dramatically reduced.

The other aim of the present work was to investigate our newly-synthesized, water-soluble CD multimers as host molecules for adducts containing Gd(III) chelates. The resulting complexes should find application as contrast agents (CAs) for MRI diagnostic medical investigations. As they have much larger molecular masses than the CAs themselves, they should be endowed with markedly higher relaxivities owing to their longer rotational correlation times and consequently generate better-contrasted images.

Results and Discussion

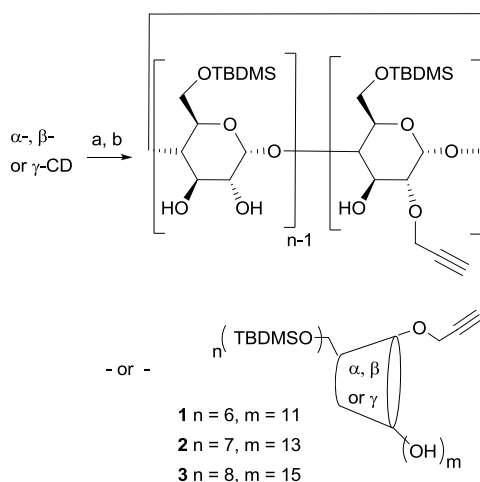
Our first goal was to prepare a library of CD dimers and trimers whose CD units would be joined 'head-to-head' or 'head-to-tail' through a rigid, chemically stable spacer. The second goal was to investigate their ability to host in their supramolecular cavities, and thus firmly bind, CAs bearing suitable substituents (e.g. one or more cyclohexyl groups; see Figure 1).

The key synthetic step was the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition by which monoazido and monoacetylenic CD derivatives could be efficiently linked together through a 1,2,3-triazole moiety. Our speedy MW-promoted protocol was characterized by short reaction times, good yields and easy work-up.

We adopted two alternative strategies: the first was to directly react two CD derivatives bearing an azido and an alkyne group respectively; the second one used a bifunctional spacer - either 1,3-bis(azidomethyl)benzene or 1,3-bis(propargyloxy)benzene - to join together two appropriate CD derivatives by generating two triazole bridges. Scheme 1 resumes the preparation of monopropargyl derivatives. 6-*O-t*-butyldimethylsilyl- β CD,²⁴ when reacted under reflux in anhydrous THF with a quasi-stoichiometric amount of propargyl bromide (1.1 eq) in the presence of lithium hydride, yielded

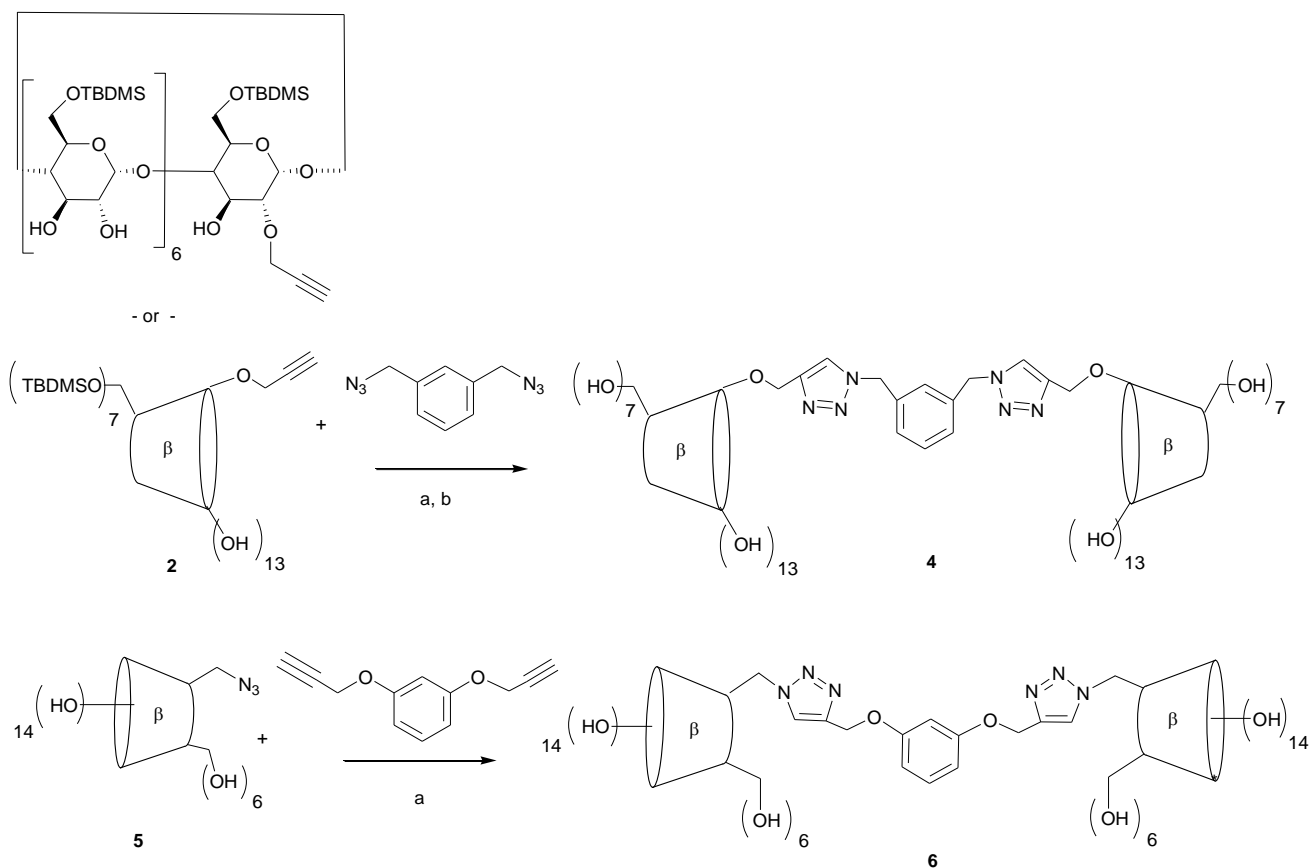
the 2-monoalkynyl derivative and traces of the 3-alkylated product. When a moderate excess of bromoalkyne (3 eq) was used instead, the dialkylated CD was isolated as major product.

6-Monoazido- β CD and 3-monoazido- α -, β - and γ CD (**5** and **10-12** respectively) were prepared as recently described by ourselves^{20,21} and so was 6^A,6^D-dideoxy-6^A,6^D-diazido- β CD (**18**), obtained by displacing with sodium azide under MW the primary (C-6) sulfonic ester group of 6^{A,D}-capped- β CD.



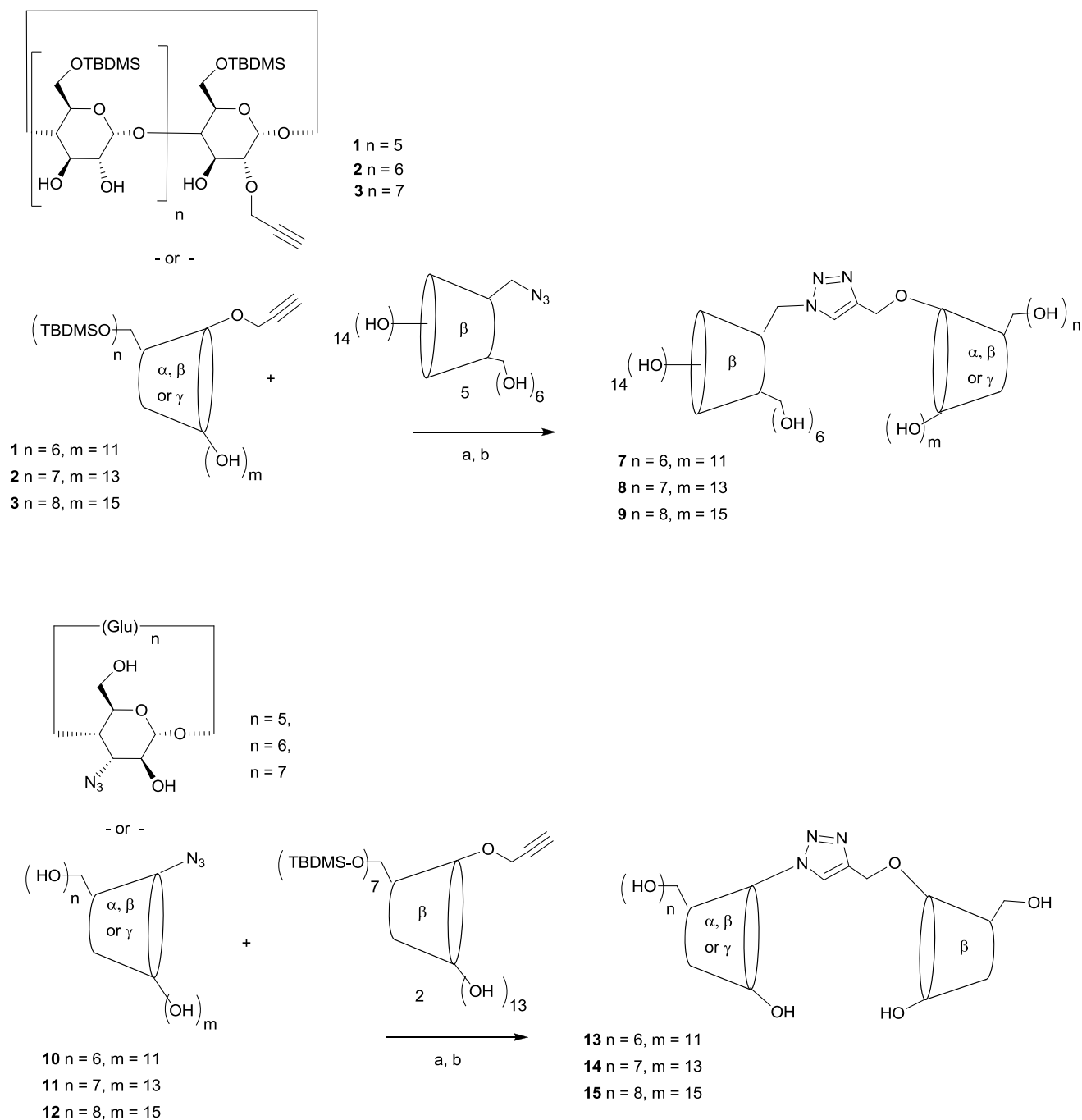
Scheme 1. Synthesis of monopropargyl CD derivatives. *Reagents and conditions:* a) TBDMSCl, imidazole, dry pyridine, stirring, rt., 8h
b) LiH, dry THF/DMSO, propargyl bromide, rfx, 4h.

Acetylenic and azido derivatives were subjected to cycloaddition in *t*-butanol/water (1:1) in the presence of CuSO₄ and L-ascorbic acid under MW (90°C, 150 W).¹⁷ The same protocol was employed to obtain symmetric homodimers (**4**, **6**) using either of the above-mentioned bifunctional cross-linkers, *viz.* 1,3-bis(azidomethyl)benzene (AMB) and 1,3-bis(propargyloxy) benzene (POB). AMB was used in our previous work to prepare cross-linked CD derivatives.²⁴



Scheme 2. Synthesis of bis-CDs and tris-CDs *via* Huisgen cycloaddition. Reagents and conditions: *a*) CuSO₄/ascorbic acid, *t*-BuOH/H₂O (1:1), MW 85°C, 150 W_{max}, 40 min; *b*) AcCl 2% in CH₃OH/CH₂Cl₂ 1:2.

When we subjected **5** to cycloaddition with a 2-mono-*O*-propargyl-6-TBDMS-CD, we obtained respectively the asymmetric homodimer **8** in the case of β CD derivative **2**, and two asymmetric heterodimers (**7**, **9**) in the case of α - or γ CD derivatives (**1**, **3**). As we aimed to compare the respective binding properties of such bis-CDs, joined by unlike faces, with those of a bis-CD that was joined by like faces, we carried out the cycloaddition on 3-monoazido- α -, β - or γ CD (**11**) and mono-*O*-alkylated- β CD (**2**).

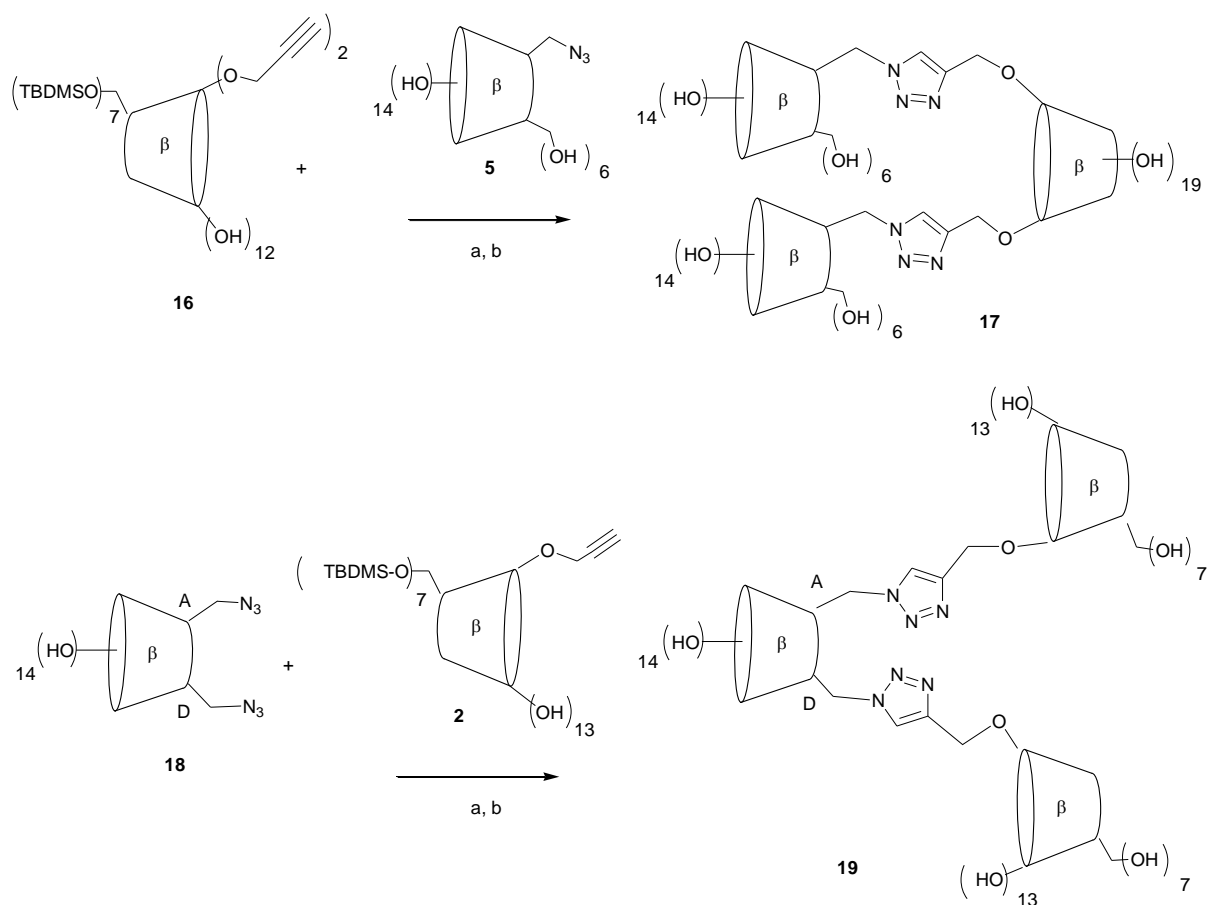


Scheme 3. Synthesis of dimers **7-15**. Reagents and conditions: *a*) CuSO₄/ascorbic acid, *t*-BuOH/H₂O (1:1), MW 85°C, 150 W_{max}, 40 min; *b*) AcCl 2% in CH₃OH/CH₂Cl₂ 1:2

We then attempted the synthesis of a tris-CD from dipropargylated derivatives (**16**) and 6-monoazido-β-CD (**5**), but found it more difficult than those of analogous bis-CDs. The operating conditions used for the latter, tended here to result in lower yields. Indeed, when carried out in *t*-butanol/water, the reaction stopped short at the dimer intermediate, that precipitated out of the medium; in fact one acetylenic group had quite failed to react. When the reaction however was repeated in DMF, the final

product (**17**) was isolated in 68% yield. Because di-*O*-alkylation generated different positional isomers, the configuration of our tris-CD is unknown.

On the other hand when 6^A,6^D-dideoxy-6^A,6^D-diazido-βCD (**18**) and mono-*O*-propargyl-6-TBDMS-β-CD (**2**) were reacted in DMF, the product was identifiable as 6^A,6^D-bis((4-(6'-*O*-hepta-TBDMS-βCD-2'-yl)-1*H*-1,2,3-triazol-1-yl)-6^A,6^D-dideoxy-βCD (**19**).



Scheme 4. Synthesis of trimers **17** and **19**. Reagents and conditions: a) CuSO₄/ascorbic acid, DMF, MW 85°C, 150 W_{max}, 40 min; b) AcCl 2% in CH₃OH/CH₂Cl₂ 1:2

Residual copper was removed from crude reaction mixtures by filtration through a silica column and crystallisation or, more efficiently, by chelation with diethylenetriamine-pentaacetic acid sodium salt. Protecting silyl groups were cleaved off **17** and **19** in a few minutes under acidic conditions (2% acetyl chloride in methanol). The final products are shown in Scheme 4.

We studied how our CD dimers and trimers interacted with suitably functionalized CAs for diagnostic Magnetic Resonance Imaging (MRI),²⁵ *e.g.* the complexes shown in Figure 1. Ditopic guest molecules can be expected to bind to bis-CDs more strongly than monotopic ones. The efficacy of a Gd(III) chelate as CA for MRI is related to its relaxivity (*r*_{1p}), that can be much enhanced by attaching it to a

very large molecule such as a bis-CD.¹⁵ To minimize its toxicity, the resulting adduct should be non-covalent rather than covalent in nature, a condition that can be satisfied if the Gd(III) chelate contains suitable hydrophobic groups that will behave as guests in the CD cavities. Relative binding parameters listed in Table 1 (association constants K_a , relaxivities R_b of the adducts and number of binding sites n), were determined by the Proton Relaxivity Enhancement (PRE) method (see supporting information for relevant equations)²⁶, which exploits the increase in relaxation rate of the paramagnetic complex that is determined by its binding to a macromolecular substrate. The binding parameters were obtained from two different kinds of titration: the first, in which a fixed concentration of the Gd-chelate was titrated with variable amounts of CD dimer/trimer (see example in Figure 2), allowed an accurate determination of K_a and R_b ; by the second, in which a fixed concentration of the CD dimer/trimer was titrated with the Gd-chelate (see example in Figure 3), we determined the number of equivalent, independent binding sites. In the latter, as the binding affinity was sufficiently high, the plot of the experimental data showed a breaking point corresponding to the substrate saturation. By fitting the experimental points with suitable PRE equations we obtained the number of binding sites on the CD dimer/trimer. While for the monotopic Gd-1 complex the number of binding sites was found to be 1 for all the investigated CDs, for bi- and tri-topic Gd-2 and Gd-3 complexes the number of binding sites on the CD dimer/trimer turned out to be fractional (0.5-0.7). Further support to these findings emerged when the data were displayed in the form of Scatchard Plots (Figure 4), where the intercept on the x axis yields the number of binding sites on the substrate.²⁷ To explain this difference we hypothesized that, while in the case of a monotopic Gd-1 complex a simple 1:1 host-guest adduct was formed, in the case of ditopic and tritopic complexes three or four dimer molecules might be bridged through two or three Gd complexes ($n=2/3$ and $n=3/4$ respectively) (Figure 7).

Figure 1. Structures of Gd complexes used to prepare host-guest adducts.

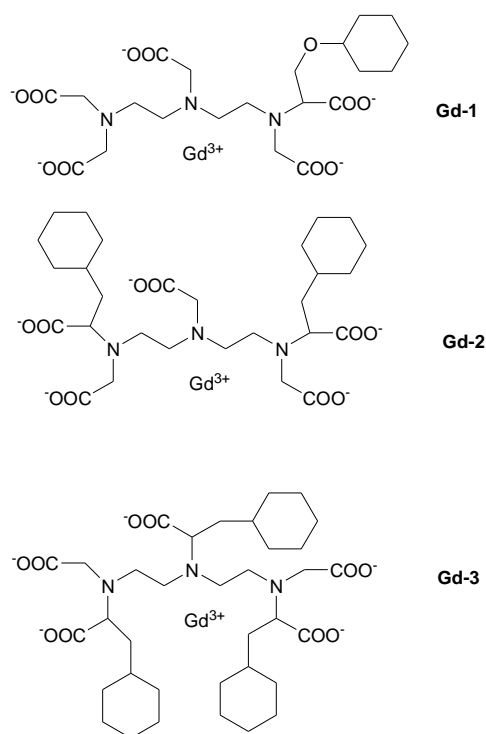


Figure 2. Observed water-proton relaxation rates of 0.53 mM **Gd-1** (▲), 0.37 mM **Gd-2** (■) and 0.48 mM **Gd-3** (★) solutions as a function of dimer **4** concentration (20 MHz, 298K, pH = 7).

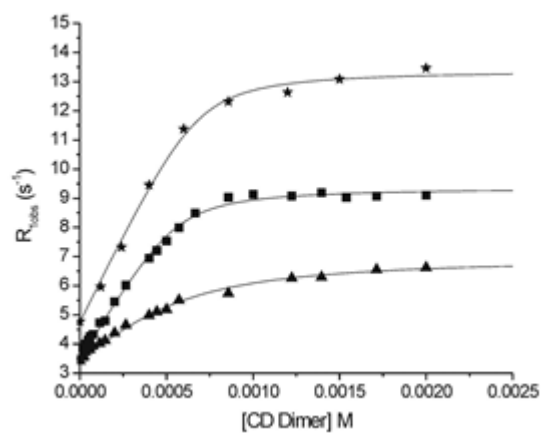


Figure 3. Observed water-proton relaxation rates of a 0.75 mM Dimer **4** solution as a function of **Gd-2** concentration (20 MHz, 298K, pH = 7).

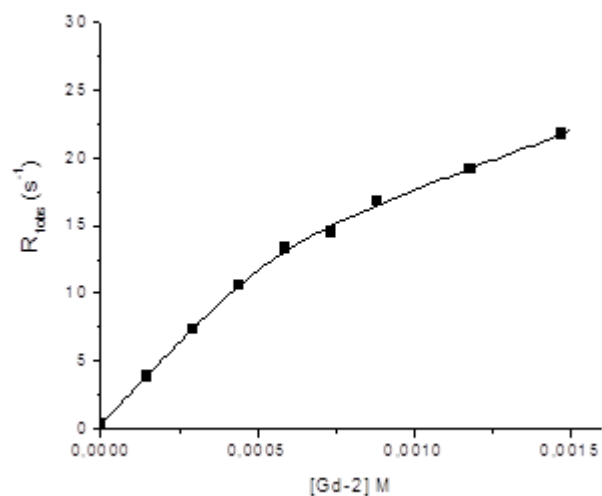
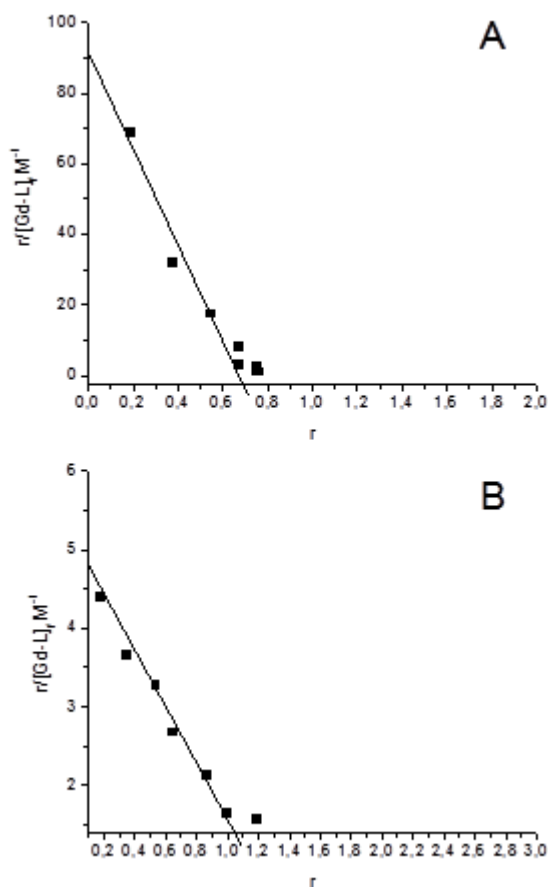


Figure 4. Scatchard plots of data obtained from PRE titrations of: A) 0.75 mM Dimer **4** solution with **Gd-2** and B) 0.375 mM dimer **4** solution with **Gd-1**. The graphs clearly point to $n=0.7$ and to $n=1$ for **Gd-2** and **Gd-1** respectively.



From inspecting Table 1 we can clearly infer a relationship between binding parameters and structural features of the interacting compounds. For example, by comparing the interactions between dimer **4** and Gd-1, Gd-2, and Gd-3 respectively (Fig. 2), we see that an increase in the number of hydrophobic cyclohexyl substituents determines an enhancement in the relaxivity of the adducts; on the other hand the affinity constant increases only when going from one to two substituents, whereas the addition of a third one does not influence it further. A closer investigation of parameters governing the relaxometric behaviour of the three CD-dimer adducts has been performed by analysing the relative NMRD (Nuclear Magnetic Resonance Dispersion) profiles.²⁸ This approach accurately determines the

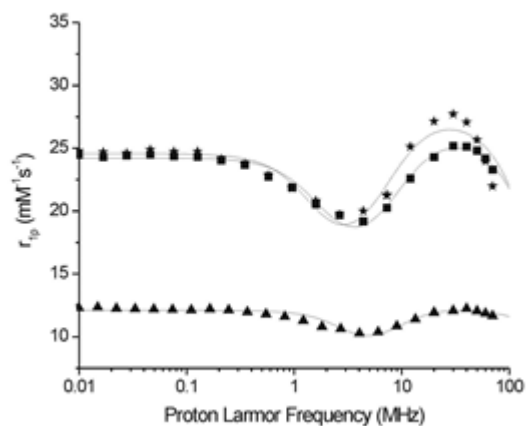
reorientational correlation time (τ_R)²⁹ of each adduct, which is strictly related to its molecular dimensions. Figure 5 shows the NMRD profiles of the adducts of dimer **4** with Gd-1, Gd-2 and Gd-3. Data were analysed using the Solomon-Bloembergen-Morgan model,³⁰ assuming (on the basis of what is observed with free complexes) one water molecule in the inner coordination sphere for each Gd(III) complex ($q=1$) and fixing the exchange lifetime (τ_M) to 200 ns. Both the profile shapes and τ_R values determined from the fitting of experimental results (Table 1) may be explained by the formation of supramolecular systems of increasing size as we go from Gd-1/dimer 4 to Gd-2/dimer 4 and Gd-3/dimer 4 inclusion complexes.

Table 1.

	Gd-1	Gd-2	Gd-3
Dimer 4	$K_a = 4.6 \times 10^3 \pm 130 \text{ M}^{-1}$ $R_b = 12.4 \pm 0.20 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$	$K_a = 3.9 \times 10^4 \pm 207 \text{ M}^{-1}$ $R_b = 24.3 \pm 0.30 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.66$	$K_a = 4.1 \times 10^4 \pm 250 \text{ M}^{-1}$ $R_b = 27.2 \pm 0.25 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.7$
	NMRD: $\tau_R = 356 \pm 12 \text{ ps}$ $n_{ss} = 0$ --	NMRD: $\tau_R = 890 \pm 17 \text{ ps}$ $n_{ss} = 6 \pm 1$ $\tau_{ss} = 60 \text{ ps}$	NMRD: $\tau_R = 930 \pm 23 \text{ ps}$ $n_{ss} = 6 \pm 0.8$ $\tau_{ss} = 60 \text{ ps}$ --
Dimer 6	$K_a = 5.9 \times 10^3 \pm 85 \text{ M}^{-1}$ $R_b = 11.5 \pm 0.45 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$ --	$K_a = 5.9 \times 10^3 \pm 115 \text{ M}^{-1}$ $R_b = 25.8 \pm 0.18 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.66$	
Dimer 7	-- --	$K_a = 1.4 \times 10^4 \pm 97 \text{ M}^{-1}$ $R_b = 21.7 \pm 0.31 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.5$	--
Dimer 8	$K_a = 1.1 \times 10^4 \pm 112 \text{ M}^{-1}$ $R_b = 10.8 \pm 0.2 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$	$K_a = 6.1 \times 10^5 \pm 223 \text{ M}^{-1}$ $R_b = 26.1 \pm 0.18 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.66$	--
Dimer 9	$K_a = 1.8 \times 10^3 \pm 88 \text{ M}^{-1}$ $R_b = 11.8 \pm 0.11 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$ --	$K_a = 3.0 \times 10^3 \pm 103 \text{ M}^{-1}$ $R_b = 27.2 \pm 0.26 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.5$	--
Dimer 14	-- --	$K_a = 1.8 \times 10^4 \pm 90 \text{ M}^{-1}$ $R_b = 24.3 \pm 0.43 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.66$	--
Trimer 17	$K_a = 1.4 \times 10^4 \pm 95 \text{ M}^{-1}$ $R_b = 12.8 \pm 0.14 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$	$K_a = 5.3 \times 10^4 \pm 135 \text{ M}^{-1}$ $R_b = 27 \pm 0.18 \text{ mM}^{-1}\text{s}^{-1}$ $n=0.7$	$K_a = 6.1 \times 10^3 \pm 112 \text{ M}^{-1}$ $R_b = 35.6 \pm 0.26 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$
	NMRD: $\tau_R = 390 \pm 88 \text{ ps}$ $n_{ss} = 0$ --	NMRD: $\tau_R = 1 \pm 0.23 \text{ ns}$ $n_{ss} = 5 \pm 1$ $\tau_{ss} = 60 \text{ ps}$	NMRD: $\tau_R = 2 \pm 0.34 \text{ ns}$ $n_{ss} = 9 \pm 1.5$ $\tau_{ss} = 60 \text{ ps}$
Trimer 19	-- -- --	$K_a = 1.9 \times 10^4 \pm 113 \text{ M}^{-1}$ $R_b = 24 \pm 0.9 \text{ mM}^{-1}\text{s}^{-1}$ $n=1$	--

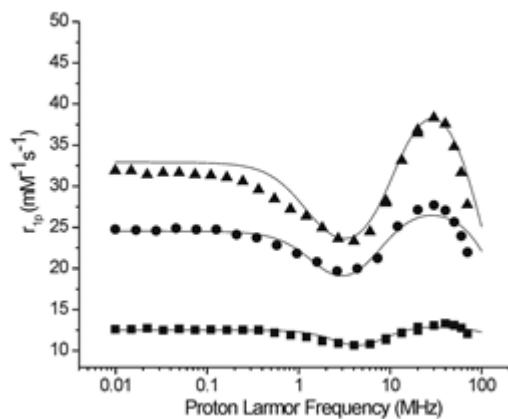
K_a = Association constant, R_b = Adduct Relaxivity, n = number of binding sites on the dimer) determined by the relaxometric PRE method at 298K and neutral pH; Reorientational correlation times (τ_R), number of second sphere water molecules (n_{ss}) and second sphere correlation time (τ_{ss}).

Figure 5.



$1/T_1$ NMRD profiles of Gd-1/Dimer 4 (▲), Gd-2/Dimer 4 (■) and Gd-3/Dimer 4 (★) adducts, as determined at pH=7 and 298K. Solid curves through data points were calculated with parameters reported in Table 1. The fitting procedure was been performed by assuming: q (number of coordinated water molecules) = 1; r (distance between the GdIII dev'essere pedice ion and the protons of the coordinated water molecule)= 3.1Å; a (distance between the GdIII ion and the outer-sphere water proton nuclei = 3.8Å; D (solute–solvent diffusion coefficient) = $2.247 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$; τ_M (exchange lifetime of the inner-sphere water molecule) was fixed to the value previously obtained for the free complexes (200ns).

Figure 6.

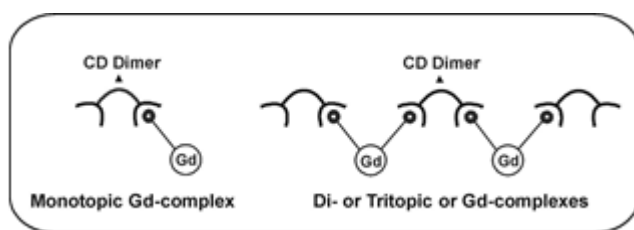


$1/T_1$ NMRD profiles of Gd-1/Trimer **17** (■), Gd-2/Trimer **17** (●) and Gd-3/Trimer **17** (▲) adducts, obtained at pH=7 and 298K. Solid curves through the data points were calculated with the parameters reported in Table 1. The fitting procedure was performed by assuming: q (number of coordinated water molecules)= 1; r (distance between the GdIII ion and the protons of the coordinated water molecule)= 3.1Å; a (distance between the GdIII ion and the outer-sphere water proton nuclei = 3.8Å; D (solute-solvent diffusion coefficient) = $2.247 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$; τ_M (exchange lifetime of the inner-sphere water molecule) was fixed to the value previously obtained for the free complexes (200ns).

For adducts of dimer **4** with Gd-2 and Gd-3, quantitative analysis of the NMRD profiles was not satisfactory when the simple inner-outer-sphere model was used; thus we assumed that the water protons present in the chelate second-coordination sphere also contributed to observed relaxivities. This contribution may originate from clusters of water molecules entrapped within the supramolecular adducts. Such a behaviour is commonly encountered with adducts formed when a paramagnetic complex is non-covalently bound at the surface of a protein²⁸ as well as with the supramolecular “host-guest” adduct formed by poly- β CD and Gd(III) complexes.³¹

From the fitting of experimental data we estimated that 5-6 second-sphere water molecules are present per Gd(III) ion (see Table 1), at an average distance of 4 Å from each paramagnetic center. This second-sphere contribution was analysed on the basis of the Solomon-Bloembergen-Morgan model, suitably modified by introducing a generic correlation time (τ^{ss}) which dealt with modulating the dipolar interactions (exchange and/or rotation) of the second-coordination-sphere water molecules. The τ^{ss} values obtained from fitting the experimental NMRD profiles appear to be much shorter than τ_R (Table 1), suggesting that they modulate the lifetimes of water molecules lying at the surface of the paramagnetic complex. Moreover, the mere contribution from the second coordination sphere does not suffice to explain the high relaxivity values of Gd-2/dimer **4** and Gd-3/dimer **4** adducts compared to the Gd-1/dimer **4** adduct. This considerable relaxivity enhancement gives strong support to the hypothesis that ditopic and tritopic complexes may act as bridges linking two or three dimer molecules, resulting in larger supramolecular adducts endowed with longer reorientational correlation times and consequently higher relaxivities (Fig. 7).

Figure 7. Hypothetic interaction mode in Gd-1/, Gd-2/ and Gd-3/dimer adducts.



Some further considerations may be appropriate regarding the association constants (Table 1) determined for the interactions of the Gd-2 complex with the other dimers here reported.

By comparing the binding properties of Gd-2 towards dimers **4** and **6**, in which the two CD units are linked by the same spacer which is however fastened to different positions on the CD surfaces, we find that the association constant is one order of magnitude larger when the two CD units are joined by their larger faces.

In the case of asymmetric heterodimers **7**, **8** and **9** interacting with Gd-2, a markedly larger association constant was found when both CDs were β . The binding affinity determined for this particular system was one order of magnitude larger than those found for all other systems.

Moreover, when we compare the binding properties of Gd-2 toward a bis-CD joined by unlike faces (dimer **8**) with those shown toward a bis-CD joined by like faces (dimer **14**), we see that the affinity is one order of magnitude lower in the latter case. These differences in the affinity constant are certainly due to the different dimensions relative orientations and of the two CD cages. For this type of guest molecules, in which the interacting unit is cyclohexane, the best cavity size is offered by β CD and the best orientation is presented by the dimer in which the CD units are joined by unlike faces.

Finally we investigated the binding properties of the three complexes reported in Figure 5 with CD trimers **17** and **19**. The strenght of interaction seems to remain in the same order of magnitude as found in the case of dimeric systems, but with Gd-3/trimer complexes relaxivity values turned out to be even higher. The NMRD profiles of Gd-1, Gd-2 and Gd-3 adducts with trimer **17** are reported in Figure 6. Their higher relaxivity values (compared to those of dimeric adducts) are supported by longer τ_R values resulting from the larger dimensions of these supramolecular systems.

Conclusions

A new approach to the preparation of CD dimers and trimers has been introduced. It employs CDs that are regioselectively monosubstituted with acetylenic and azido groups, or, alternatively, the same in association with disubstituted aromatic spacers bearing either reactive function. These versatile derivatives were subjected to a MW-promoted Huisgen cycloaddition that led to the formation of bis- and tris-CDs. Access to a library of CD dimers and trimers featuring different linkage positions on CD surfaces and cavities of different dimensions should enable us to better tune the interaction between the guest molecule (drug, diagnostic marker, etc.) and the CD carrier. Our study with medical diagnostic markers (MRI contrast agents) as guest molecules bore out this concept. Moreover stability constants and relaxivities found for the adducts of Gd(III) chelates with our CD multimers, especially trimers, recommend these supramolecular systems as promising candidates for MRI applications.

Experimental

General

Materials and methods: Reactions were monitored by TLC on Merck 60 F₂₅₄ (0.25 mm) plates, which were visualized by UV inspection and/or by heating after a spray with 5% H₂SO₄ in ethanol. Merck silica gel was used for column chromatography (CC). IR spectra were recorded with a Shimadzu FT-IR 8001 spectrophotometer. Unless stated otherwise, NMR spectra were recorded with a Bruker 300 Advance (300 MHz and 75 MHz for ¹H and ¹³C, respectively) at 25°C; chemical shifts are calibrated to the residual proton and carbon resonances of the solvent: CDCl₃ ($\delta_{\text{H}} = 7.26$, $\delta_{\text{C}} = 77.0$). Chemical shifts (δ) are given in ppm, coupling constants (J) in Hz. MALDI-TOF MS spectra were recorded on a Bruker Reflex III spectrometer, ESI-mass spectra on a Waters Micromass ZQ equipped with ESI source. MW-promoted reactions were carried out in a professional oven from Microsynth - Milestone (Italy). Commercially available reagents and solvents were used without further purification unless otherwise noted. Native CDs were kindly provided by Wacker Chemie. Gd1-3 (Figure 1) were kindly provided by Bracco Imaging Spa (Italy).

Synthesis

2^I-*O*-propargyl-6^{I,VII}*O*-(*t*-butyldimethylsilyl)- β CD (**2**), 6^I-azido-6^I-deoxy- β CD (**5**) and 3^I-deoxy-3^I-*altro*-azido- α -, β - and γ CD (**10**, **11**, **12**) and biphenyl-4,4'-disulfonyl-A,D-capped β CD were prepared following published procedures.^{20,22,32} *O*-propargyl derivatives of α - and γ CD (**1**, **3**) had not so far been described in the literature.

2^I-propargyl-6^{I,VII}-*O*-TBDMS- α CD (**1**)

6^{I,VII}-*O*-TBDMS- α CD (3 g, 1.8 mmol) and LiH (43 mg, 5.4 mmol) were dissolved in anhydrous THF (25 mL). The solution was heated 2 h under reflux. After it had cooled down to room temperature, 80% propargyl bromide in toluene (292 μ L, 2 mmol) dissolved in 2 mL of THF was added dropwise and the mixture was stirred overnight at room temperature. The reacted mixture was diluted with EtOAc, washed with 1M H₂SO₄ and brine, and finally dried (Na₂SO₄). The crude residue, purified by CC (CHCl₃–acetone) yielded 1.35 g of derivative **1** (0.8 mmol, yield 44%).

1 is a white powder; R_f = 0.43 (CHCl₃/CH₃OH 4:1); ν_{\max} (KBr)/cm⁻¹ 3420, 2930, 1473, 1361, 1084, 1041 and 835; δ_H (300 MHz; CDCl₃) 4.5 (br s, 6H, 1-H), 4.04 (br d, J 9.4, 1H, 1'-H), 3.98 (br d, J 9.4, 1H, 1'-H), 4.0-3.49 (m, 36H), 2.43 (br. s, 1H, 3'-H), 1.08 (s, 54H, *t*-but) and 0.05 (s, 36H, Si-CH₃); m/z (ESI-MS) calcd. for [M+H]⁺ 1697.4, found 1697.3.

2^I-propargyl-6^{I,VII}-*O*-TBDMS- γ CD (**3**)

6^{I,VII}-*O*-TBDMS- γ CD (3 g, 1.35 mmol) and LiH (30 mg, 3.75 mmol) were dissolved in anhydrous THF (25 mL) and the solution was heated 2 h under reflux. After it had cooled down to room temperature, 80% propargyl bromide in toluene (221 μ L, 1.48 mmol), diluted in 2 mL of THF, was added dropwise and the mixture was stirred overnight at room temperature. The reacted mixture was diluted with EtOAc, washed with 1M H₂SO₄ and brine, and finally dried (Na₂SO₄). The crude residue, purified by CC (CHCl₃-Acetone) yielded 1.57 g of derivative **3** (0.702 mmol, yield 52%).

3 is a white powder; R_f = 0.25 (CHCl₃/CH₃OH 4:1); ν_{\max} (KBr)/cm⁻¹ 3420, 2930, 1471, 1362, 1086, 1035 and 835; δ_H (300 MHz; CDCl₃) 5.1-4.9 (m, 8H, 1-H), 4.78 (br d, J 8.1, 2H, 1'-H), 4.11-3.40 (m, 48H), 2.1 (br. s, 1H, 3'-H), 0.9 (s, 72H, *t*-but) and 0.05 (s, 48H, Si-CH₃); m/z (MALDI-TOF MS) calcd. for [M+Na]⁺ 2270.120, found 2270.148.

Di-*O*-propargyl-6^{I,VII}-*O*-TBDMS- β CD (**16**).

6^{I,VII}-*O*-TBDMS- β CD (3 g, 1.55 mmol) and LiH (37 mg, 4.6 mmol) were dissolved in anhydrous THF (12 mL). The solution was heated 2 h under reflux. After it had cooled down to room temperature, 80% propargyl bromide in toluene (690 μ L, 4.65 mmol), diluted in 2 mL of THF, was added dropwise and the mixture was stirred overnight at room temperature. The reacted mixture was diluted with

EtOAc, washed with 1M H₂SO₄ and brine, and finally dried (Na₂SO₄). The crude residue, purified by CC (CHCl₃-CH₃OH 19:1, 9:1, 4:1) yielded 1.24 g of di-*O*-alkylated-βCD (0.62 mmol, yield 40%).

16 is a white powder; *R_f* = 0.26 (CHCl₃/CH₃OH 4:1); *v*_{max}(KBr)/cm⁻¹ 3420, 3325, 1473, 1254, 1086, 1040 and 835; *δ*_H(300 MHz; CDCl₃) 4.9 (br s, 7H, 1-H), 4.5 (br q, *J* 16.8, 4H, 1'-H), 4.1-3.9 (m, 14H), 3.8-3.5 (m, 28H), 2.4 (br. t, *J* 2.3, 2H, 3'-H), 0.88 (s, 63H, *t*-but) and 0.05 (s, 42H, Si-CH₃); *m/z* (MALDI-TOF MS) calcd. for [M+Na]⁺ 2031.996, found 2032.322.

6^A,6^D-diazido-6^A,6^D-dideoxy-βCD (18).

The reaction was carried out under magnetic stirring in a professional MW oven, temperature being monitored with a fibre-optic thermometer. 500 mg (0.35 mmol) of biphenyl-4,4'-disulfonyl-A,D-capped βCD and 66 mg (1.05 mmol) of sodium azide were dissolved in 5 ml of DMF. The mixture was irradiated with MW (120W) at 85°C for 40 min. The solvent was then partially evaporated and the product precipitated with 7 ml of water/acetone 1:10. 403 mg of pure 6^A,6^D-diazido-6^A,6^D-dideoxy-βCD were recovered (yield 96 %). Analytical data were in accordance with reported values.³³

General procedure for Cu-catalyzed Huisgen 1,3-dipolar cycloadditions

In a 10 mL two-necked round-bottomed flask (equipped with an optical-fiber thermometer for reactions under MW) 1 mmol of the alkyl azide and 1 mmol of the acetylenic derivative were suspended in 5 mL of a *t*-BuOH/H₂O 1:1 mixture. 0.6 mmol of CuSO₄·5H₂O and 1.2 mmol of L-ascorbic acid were added. The mixture was irradiated with MW at constant temperature (90°C, max power 150 W) and the reaction monitored by TLC until complete conversion of the starting material was observed. Water (30 mL) was then added; the precipitate was filtered off and washed with a cold 40 mM solution of diethylenetriamine-pentaacetic acid sodium salt (20 mL) to remove copper, and finally with water.

General procedure for deprotection.

The crude product (1 mmol) was dissolved in CH₂Cl₂ (20 mL), a 2% solution of AcCl in MeOH (10 mL) was added and the mixture was stirred overnight at room temperature. Ether (50 mL) was then added; the precipitate was filtered, washed with ether (40 mL) and dried under vacuum.

1,3-bis((4-(βCD-2'-yl-methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzene (4).

The reaction was carried out with 2^I-*O*-propargyl-6^{I-VII}-*O*-TBDMS-βCD (**2**) (500 mg, 0.25 mmol) and 1,3-bis(azidomethyl) benzene (AMB, 23 mg, 0.12 mmol). The reacted mixture was diluted with EtOAc, washed with H₂O and brine and finally dried (Na₂SO₄). The crude product, when purified by CC (CHCl₃/CH₃OH = 19:1, 9:1, 4:1), yielded 370 mg of 6^{I-VII}TBDMS β-β dimer (0.09 mmol, yield 75%) as a white powder. *R_f* = 0.43 (CHCl₃/CH₃OH 4:1) and 0.56 (EtOAc/MeOH/H₂O = 40:7:5); *v*_{max}(KBr)/cm⁻¹ 3422, 1473, 1389, 1254, 1086, 1040 and 835; *δ*_H(300 MHz; CDCl₃) 7.9 (s, 2H, 5-H *triazole*), 7.4 (m, 1H, 5-H), 7.35-7.3 (m, 3H, 2-H, 4-H, 6-H), 5.53 (s, 4H, -N-CH₂-Ph), 4.9 (m, 18H, 1'-H, overlapped 4 H, *triazole*-CH₂-O), 4.09-3.9 (m, 28H), 3.7-3.4 (m, 56H), 0.88 (s, 126H, *t*-but), 0.05 (s, 84H, Si-CH₃); *δ*_C(75 MHz; CDCl₃) 145.1 (C-4 *triazole*), 135.8 (C-1,3), 130.1 (C-5), 128.8, 128.2, 128.0 (C-2,C-4,C-6), 124.2 (C-5 *triazole*), 102.7 (C-1'), 82.1 (C-4'), 73.8, 73.4, 73.2 (C-2',C-3',C-5'), 65.3 (C *triazole* -CH₂-O), 62.1 (C-6'), 54.7 (C N-CH₂-Ph), 26.4 (C-Me₃), 18.7 (C-Me₃), -4.0, -4.2 (Si-Me₂); *m/z* (MALDI-TOF MS) calcd. for [M+Na]⁺ 4153.053, found 4152.388. 370 mg of 6^{I-VII}TBDMS β-β dimer (0.09 mmol) were deprotected to obtain 180 mg of β-β dimer **4** (180 mg, 0.07 mmol, yield 80%).

4 is a white powder. *R_f* = 0.51 (CH₃CN/H₂O 2:1); *v*_{max}(KBr)/cm⁻¹ 3435, 1490, 1399, 1257, 1090, 1050 and 852; *δ*_H(300 MHz; D₂O) 7.9 (s, 2H, 5-H *triazole*), 7.3 (t, *J* 7.9, 1H, 5-H), 7.2 (d, 2H, *J* 7.9, 4,6-H), 7.1 (s, 1H, 2-H), 5.53 (s, 4H, -N-CH₂-Ph), 4.9 (m, 14H, 1'-H), 4.7 (m, 4H, *triazole*-CH₂-O), 3.9-3.7 (m, 56H), 3.6-3.4 (m, 24H), 3.4 (dd, *J* 9.9 and 3.6, 2H, 2'-H), 3.2 (t, *J* 9.9, 2H, 4'-H); *δ*_C(75 MHz; D₂O) 144.2 (C-4 *triazole*); 136.1 (C-1,C-3), 130.4, 128.7, 128.6, 127.9 (C-2,C-4,C-5,C-6), 126.0 (C-5 *triazole*), 102.2 (C-1'), 81.8 (C-4'), 73.8, 73.4, 73.2 (C-2',C-3',C-5'), 65.3 (C *triazole*-CH₂-O), 60.6 (C-6'), 54.1 (N-CH₂-Ph); *m/z* (MALDI-TOF MS) calcd. for [M+Na]⁺ 2555.842, found 2556.074.

1,3-bis((1-(6'-deoxy-βCD-6'-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzene (**6**).

The reaction was carried out with 6^I-azido-6^I-deoxy-βCD (**5**) (400 mg, 0.34 mmol) and 1,3-bis(propargyloxy)benzene (POB) (23 mg, 0.12 mmol). Acetone (30 ml) was added to the reacted mixture and the precipitate was filtered off. The solid was recrystallised from water/acetone 1:2 and 215 mg of pure β,β dimer **6** were recovered (0.084 mmol, yield 70%).

Analytical data were in accordance with reported values.³⁴

6 is a white powder. *R_f* = 0.2 (CH₃CN/H₂O 2:1); *v*_{max}(KBr)/cm⁻¹ 3422, 1640, 1389, 1254, 1040 and 835; *δ*_H(300 MHz; D₂O) 7.7 (s, 2 H, 5-H *triazole*), 7.2 (t, *J* 6.8, 1H, 5-H), 7.1 (s, 1 H, 2-H), 6.8 (d, *J* 6.8, 1H, 4-H), 6.6 (d, *J* 6.8, 1H, 6-H), 5.3-4.8 (m, 18H, 1'-H overlapped 4 H, *triazole*-CH₂-O), 4.0-3.0 (m, 80H), 3.0 (m, 2H), 2.9 (m, 2H); *m/z* (ESI-MS) calcd. for [M+Na₂]²⁺ = 1253.4, found 1253.7.

2-(*O*-Di-(1-(6'-deoxy- β CD-6'-yl)-1*H*-1,2,3-triazol-4-yl)methyl)- β CD (**17**).

The reaction was carried out in DMF with di-2^I-*O*-propargyl-6^{I-VII}-*O*-TBDMS- β CD (**2**) (414 mg, 0.2 mmol) and 6^I-azido-6^I-deoxy- β CD (**5**) (525 mg, 0.45 mmol). Acetone (30 ml) was added to the reacted mixture, the precipitate was filtered off, washed with a cold 40 mM solution of diethylenetriamine-pentaacetic acid sodium salt (20 mL) to remove copper, and finally with water. The 6^{I-VII}TBDMS β - β trimer is a white powder (618 mg, 0.14 mmol, yield 68%). $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3422, 1474, 1364, 1254, 1082, 1038 and 835; $\delta_{\text{H}}(300 \text{ MHz}; \text{DMSO-}d_6)$ 8.0 (s, 2H, 5-H *triazole*), 5.1-4.6 (m, 25H, 1-H overlapped 4 H, triazole-CH₂-O), 3.8-3.2 (m, overlapped with H₂O), 0.88 (s, 63H, *t*-but), 0.04 (s, 42H, Si-CH₃); m/z (MALDI-TOF MS) calcd. for [M+Na]⁺ 4350.749, found 4350.952. Starting with 6^{I-VII}TBDMS β - β trimer (618 mg, 0.143 mmol), we obtained trimer **17** (290 mg, 0.082 mmol, yield 58%) as a white powder. $R_f = 0.06$ (CH₃CN/H₂O 2:1); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3422, 1465, 1380, 1270, 1095, and 850; $\delta_{\text{H}}(300 \text{ MHz}; \text{D}_2\text{O})$ 8.0 (s, 1H, 5-H *triazole*), 5.2-4.9 (m, 25H, 1'-H overlapped 4 H triazole-CH₂-O), 4.1 (br t, J 7.5, 2H), 3.9-3.2 (m, 120H), 3.1 (m, 2H), 2.8 (m, 2H); $\delta_{\text{C}}(75 \text{ MHz}; \text{D}_2\text{O})$ 144.3 (C-4 *triazole*) 127.3 (C-5 *triazole*), 102.3-101.8 (C-1'), 81.7-81.5 (C-4'), 73.5-73.0 (C-5'), 72.4-71.8 (C-2', C-3'), 69.8 (C triazole-CH₂-O), 61.3-60.5 (C-6'); m/z (ESI-MS) m/z (MALDI-TOF MS) calcd. for [M+Na]⁺ 3552.144, found 3552.523.

6^A,6^D-bis((4-(6'- β CD-2'-yl)-1*H*-1,2,3-triazol-1-yl)-6^A,6^D-dideoxy- β CD (**18**).

The reaction was carried out with 6^A,6^D-dideoxy-6^A,6^D-diazido- β CD (**12**) (80 mg, 0.067 mmol) and 2^I-*O*-propargyl-6^{I-VII}-*O*-TBDMS- β CD (**2**) (264 mg, 0.135 mmol). The solvent was then partially evaporated and the reacted mixture was diluted with CHCl₃, washed with H₂O (3 x 20 ml), dried (Na₂SO₄) and evaporated to dryness. The crude product, purified by CC (CHCl₃/CH₃OH = , 9:1, 4:1), yielded the desired 6^{I-VII}TBDMS β - β trimer (252 mg, 0.049 mmol, 73%) as a white powder. $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3422, 1475, 1389, 1263, 1090, 1038 and 835; $\delta_{\text{H}}(300 \text{ MHz}; \text{DMSO-}d_6)$ 7.99 (1 H, s, 5-H *triazole*), 6.0-5.5 (OH, m); 5.0-4.5 (25 H, m, 1-H overlapped 4 H, triazole-CH₂-O), 4.20-3.15 (126 H, m), 0.8 (s, 126 H, *t*-but), 0.01 (s, 84 H, Si-CH₃); m/z (MALDI-TOF MS) calcd. for [M+Na]⁺ 5149.4, found 5149.4. Starting from 6^{I-VII}TBDMS β - β trimer (150 mg, 0.029 mmol), the mixture, stirred overnight at room temperature, yielded tris-CD **18** (68 mg, 0.02 mmol, yield 68%) as a white powder. $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3412, 1477, 1390, 1255, 1085, 1038 and 835; $\delta_{\text{H}}(300 \text{ MHz}; \text{D}_2\text{O})$ 8.2 (s, 1H, 5-H *triazole*), 5.3-4.4 (m, 25H, 1-H overlapped 4 H triazole-CH₂-O), 4.0-3.5 (126 H, m), 2.8 (m, 2H); $\delta_{\text{C}}(75 \text{ MHz}; \text{D}_2\text{O})$ 143.7, (C-4 *triazole*), 127.4 (C-5 *triazole*), , 102.2-101.7 (C-1'), 81.9-81.4 (C-

4'), 73.4 (C-5') 72.3-71.8 (C-2',C-3'), 66.2 (C triazole-CH₂-O), 60.6-59.3 (C6') *m/z* (MALDI-TOF MS) calcd. for [M+Na]⁺ 3552.144 found 3552.417.

Water proton relaxivity measurements

Longitudinal water-proton relaxation rates were measured by a Stellar Spinmaster spectrometer (Mede, PV- Italy) operating at 0.47 T, by means of the standard inversion-recovery technique (16 experiments, 2 scans). Typical 90° pulse width was 7.5 μs and the reproducibility of the T1 data was ±0.5%. Temperature was controlled (±0.1°C) with a Stellar VTC-91 air-flow heater equipped with a copper-constantan thermocouple. The proton 1/T1 NMRD profiles were measured over a continuum of magnetic field strength ranging from 0.00024 to 0.47 T (corresponding to 0.01-20 MHz proton Larmor Frequency) on a Stellar field-cycling relaxometer. This works under complete computer control with an absolute uncertainty in 1/T1 of ±1%. Data points from 0.47 T (20 MHz) to 1.7 T (70 MHz) were collected on a Stellar Spinmaster spectrometer

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